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NEWS 12 FEB 23 TOXCENTER updates mirror those of MEDLINE - more precise author group fields and 2009 MeSH terms  
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NEWS 21 APR 03 CAS coverage of exemplified prophetic substances enhanced  
NEWS 22 APR 07 STN is raising the limits on saved answers  
NEWS 23 APR 24 CA/CAPLus now has more comprehensive patent assignee information  
NEWS 24 APR 26 USPATFULL and USPAT2 enhanced with patent assignment/reassignment information  
NEWS 25 APR 28 CAS patent authority coverage expanded  
NEWS 26 APR 28 ENCOMPLIT/ENCOMPPLIT2 search fields enhanced  
NEWS 27 APR 28 Limits doubled for structure searching in CAS REGISTRY  
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NEWS 30 MAY 11 BELLSTEIN substance information now available on STN Easy  
NEWS 31 MAY 14 DGENE, PCTGEN and USGENE enhanced with increased limits for exact sequence match searches and introduction of free HIT display format

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=> s screen?  
L1 1749819 SCREEN?

=> s 11 and antibod?  
L2 143903 L1 AND ANTIBOD?

=> s 12 and glycospecific  
L3 4 L2 AND GLYCOSPECIFIC

=> s 13 and human TSH  
L4 0 L3 AND HUMAN TSH

=> s 12 and human TSH  
1.5 79 L2 AND HUMAN TSH

=> s 15 and recombinant  
L6 22 L5 AND RECOMBINANT

=> s 16 and sialylated  
L7 0 L6 AND SIALYLATED

=> s 16 and branched  
L8 0 L6 AND BRANCHED

=> s 16 and recombinant TSH  
L9 5 L6 AND RECOMBINANT TSH

=> dup remove 19  
PROCESSING COMPLETED FOR L9  
L10 2 DUP REMOVE L9 (3 DUPLICATES REMOVED)

=> d l10 1-2 cb1b abs

L10 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1  
1996407409. PubMed ID: 8811462. Epitope mapping of a **recombinant**  
human TSH receptor extracellular domain: identification  
of a predominant epitope using animal sera. Hunt N; Willey K P; Abend N;  
Northemann W; Leidenberger F A. (Institute for Hormone and Fertility  
Research, University of Hamburg, Germany.) Journal of clinical laboratory  
analysis, (1996) Vol. 10, No. 4, pp. 193-204. Journal code: 8801384.  
ISSN: 0887-8013. Pub. country: United States. Language: English.  
AB The extracellular domain of the TSH receptor (TSHR-561, amino acids  
#78-389) was expressed as a hexa-histidine fusion protein in bacteria.  
The **recombinant** protein was purified to homogeneity and used to  
immunize porcine and ovine species. High titre **antibodies** were  
obtained from both species that recognized the **recombinant**  
protein in Western blot analysis but failed to interfere with the TSH  
radio receptor assay. An epitope library was constructed and  
**screened** with affinity purified ovine and porcine antisera and  
detected a number of positive clones. Sequence analysis revealed that all  
of the epitopes contained sequences derived from the carboxyl terminus of  
the **recombinant** immunogen. One clone defined an epitope  
covering 16 amino acids from the carboxyl terminus and was the common  
epitope found in all of the other clones. Western blot **screening**  
of a large panel of Graves' sera with **recombinant TSH**  
receptor protein identified one patient sera that also recognized linear  
epitopes in the TSHR-561 protein. Experimentation demonstrated that the  
linear epitope recognized by this human sera was identical to the sequence  
recognised by the animal antisera. This sequence is unique to the TSH  
receptor and will be useful in further studies to analyze the TSH receptor  
protein.

L10 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2009 ACS on STN  
1990:453366 Document No. 113:53366 Original Reference No. 113:8913a,8916a  
Cloning, sequencing and expression of the human thyrotropin (TSH)  
receptor: evidence for binding of autoantibodies. Libert, Frederick,  
Lefort, Anne; Gerard, Catherine; Parmentier, Marc; Perret, Jason; Ludgate,  
Marian; Dumont, Jacques E.; Vassart, Gilbert (Fac. Med., Univ. Libre  
Brussels, Brussels, 1070, Belg.). Biochemical and Biophysical Research  
Communications, 165(3), 1250-5 (English) 1989. CODEN: BBRCA9. ISSN:  
0006-291X.

AB A human thyroid cDNA library was **screened** by hybridization with  
a dog TSH receptor (TSHr) cDNA. Sequencing of the resulting clones  
identified a 2292-residue open reading frame encoding a 744-amino acid  
mature polypeptide presenting 90.3% similarity with the dog TSHr. Two  
major transcripts (4.6 and 4.5 kb) were identified in the human thyroid  
which suggests that alternative splicing could generate multiple forms of

human TSHr. Transfection of the coding sequence in COS-7 cells conferred to a membrane preparation of these cells the ability to bind specifically TSH. TSH binding was completely displaced by Ig preps. from patients with idiopathic myxedema.

=> s 12 and human TSR  
L11 0 L2 AND HUMAN TSR

=> s 12 and TSR  
L12 4 L2 AND TSR

=> dup remove 112  
PROCESSING COMPLETED FOR L12  
L13 4 DUP REMOVE L12 (0 DUPLICATES REMOVED)

=> d l13 1-4 cbib abs

L13 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN  
2007:999439 Document No. 147:336350 Stem cell factor-like protein SCFA1, its protein and cDNA sequences, and therapeutic uses in modulating gastrointestinal and oral epithelial cell proliferation. Emtage, Peter C. R.; Funk, Walter (Nuvelo, Inc., USA). PCT Int. Appl. WO 2007100357 A2 20070907, 119pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-US39266 20061006. PRIORITY: US 2005-724908P 20051007.

AB Methods for stimulating epithelial cell proliferation and for treating oral and gastrointestinal disorders are described. The methods use compns. comprising stem cell factor-like protein A1 (SCFA1) polypeptides and polynucleotides, including human protein and cDNA sequences. SCFA1, also known as hPWTSR and R-spondin 3, is a member of the thrombospondin type 1 repeat (TSR) superfamily. SCFA1 contains predicted signal peptide, 2 furin-like domains and a thrombospondin type 1 domain (TSP1).

L13 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN  
2005:429525 Document No. 142:477114 DNA molecules and polypeptides of glycosyltransferases and enzymes involved in biosynthesis of deoxysugars from Streptomyces and Saccharopolyspora, their sequences and use in glycosylation of various small molecules, such as spinosyn. Trefzer, Axel; Green, Brian D.; Bibb, Mervyn; Mason, Dylan (Diversa Corporation, USA). PCT Int. Appl. WO 2005044979 A2 20050519, 226 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US25015 20040804. PRIORITY: US 2003-492781P 20030804; US 2003-515950P 20031029.

AB The invention provides a novel technol. for glycosylation of natural small mol. products using genetically engineered strains of bacteria. The invention relates that the in vivo glycosylation system expresses a

heterologous glycosyltransferase and enzymes involved in the biosynthesis of deoxysugars, which is capable of glycosylating a suitable substrate, which can be added to a culture broth. The invention also provides glycosylated derivs. of spinosyn, erythromycin, tetracycline, rifampicin, daunorubicin, mithramycin, rapamycin, FK520, FK506, amphotericin, tylosin, and/or avermectin using said disclosed glycosylation system and transformed bacteria. The invention further provides DNA and amino acid sequences of various glycosyltransferases and enzymes involved in biosynthesis of deoxysugars from Streptomyces and Saccharopolyspora, and the use of these in production of genetically engineered bacteria for glycosylating small mols. The invention also relates that said DNA sequences encode enzymes involved in biosynthesis of deoxysugar including enzymes having the following activities: nucleodidyl transferase, 4,6-dehydratase, 3,5-epimerase, 4-ketoreductase, 2,3-dehydratase, and 3-ketoreductase activities. Still further, the invention provides: (a) methods for producing said recombinant enzymes; (b) use of glycosylated spinosyn and pseudoaglycone derivs. in production of insecticides, disinfectants and pharmaceutical compns.; and (c) primers and probes specific for disclosed DNA sequences. Finally, the invention provides: (a) various methods for production of modified nucleic acid mols. encoding glycosyltransferases and enzymes involved in biosynthesis of deoxysugars from Streptomyces and Saccharopolyspora and (b) chimeric proteins comprising said glycosyltransferases and enzymes involved in biosynthesis of deoxysugars and a heterologous protein.

L13 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN  
2003:261865 Document No. 138:268316 Virulent gene products of *Salmonella typhimurium* and their therapeutic uses thereof. Dougan, Gordon (Microscience Limited, UK). PCT Int. Appl. WO 2003027140 A2 20030403, 20 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB4333 20020926. PRIORITY: GB 2001-23170 20010926.

AB The present invention is based on the discovery of genes in *Salmonella typhimurium*, the products of which are implicated in virulence and colonization. A comparison of genes that are inactive in a non-pathogenic microorganism but which are active in a related pathogenic microorganism has identified genes which may be used in therapy or diagnosis. Accordingly, peptides encoded by a gene from *S. typhimurium* that corresponds to any of those listed in Table 1, can be used in therapy or diagnosis. The peptides have many therapeutic uses for treating *Salmonella* infections, including use in vaccines for prophylactic application.

L13 ANSWER 4 OF 4 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN  
2000:815242 The Genuine Article (R) Number: 367YQ. Subcommissural organ/Reissner's fiber complex: Characterization of SCO-spondin, a glycoprotein with potent activity on neurite outgrowth. Meiniel A (Reprint). Fac Med, Lab Biochim Med, 28 Pl Henri Dunant, F-63001 Clermont Ferrand, France (Reprint). Gobron S; Creveaux I; Meiniel R; Didier R; Herbet A; Bamdad M; El Bitar F; Dastugue B. Fac Med, Lab Biochim Med, F-63001 Clermont Ferrand, France; INSERM, U384, Clermont Ferrand, France. GLIA (NOV 2000) Vol. 32, No. 2, pp. 177-191. ISSN: 0894-1491. Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In the developing vertebrate nervous system, several proteins of the thrombospondin superfamily act on axonal pathfinding. By successive screening of a SCO-cDNA library, we have characterized a new member of this superfamily, which we call SCO-spondin. This extracellular matrix glycoprotein of 4,560 amino acids is expressed and secreted early in development by the subcommissural organ (SCO), an ependymal differentiation located in the roof of the Sylvian aqueduct. Furthermore, SCO-spondin makes part of Reissner's fiber (RF), a thread-like structure present in the central canal of the spinal cord. This novel protein shows a unique arrangement of several conserved domains, including 26 thrombospondin type 1 repeats (**TSR**), nine low-density lipoprotein receptor (LDLr) type A domains, two epidermal growth factor (EGF)-like domains, and N- and C-terminal von Willebrand factor (vWF) cysteine-rich domains, all of which are potent sites of protein-protein interaction. Regarding the huge number of **TSR**, the putative function of SCO-spondin on axonal guidance is discussed in comparison with other developmental molecules of the CNS exhibiting **TSR**. To correlate SCO-spondin molecular feature and function, we tested the effect of oligopeptides, whose sequences include highly conserved amino acids of the consensus domains on a neuroblastoma cell line B 104. One of these peptides (WSGWSSCSRSCG) markedly increased neurite outgrowth of B 104 cells and this effect was dose dependent. Thus, SCO-spondin is a favorable substrate for neurite outgrowth and may participate in the posterior commissure formation and spinal cord differentiation during ontogenesis of the central nervous system. (C) 2000 Wiley-Liss, Inc.

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=> s 12 and carbohydrate dependent
L14          23 L2 AND CARBOHYDRATE DEPENDENT

=> s 114 and sialylated
L15          0 L14 AND SIALYLATED

=> s 114 and sialylation
L16          0 L14 AND SIALYLATION

=> dup remove 114
PROCESSING COMPLETED FOR L14
L17          5 DUP REMOVE L14 (18 DUPLICATES REMOVED)

=> d 117 1-5 cbib abs
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L17 ANSWER 1 OF 5      MEDLINE on STN          DUPLICATE 1
2008277026.  PubMed ID: 18400317.  Antigenic differences within the
Cryptosporidium hominis and Cryptosporidium parvum surface proteins P23
and GP900 defined by monoclonal antibody reactivity. Sturbaum
Gregory D; Schaefer Deborah A; Jost B Helen; Sterling Charles R; Riggs
Michael W. (CH Diagnostic & Consulting Service Inc, Berthoud, CO 80513,
USA.. gsturbaum@chdiagnostic.com). Molecular and biochemical
parasitology, (2008 Jun) Vol. 159, No. 2, pp. 138-41. Electronic
Publication: 2008-03-04. Journal code: 8006324. ISSN: 0166-6851. Pub.
country: Netherlands. Language: English.
```

AB The biological basis for the specificity of host infectivity patterns of *Cryptosporidium* spp., in particular *C. hominis* and *C. parvum*, has yet to be fully elucidated. Comparison of the *C. parvum* and *C. hominis* P23 and GP900 predicted amino acid sequences revealed 3 differences in P23 and 4 and 17 differences in GP900 domains 1 and 5, respectively. Using monoclonal **antibodies** developed against the surface (glyco)proteins P23 and GP900 of the *C. parvum* Iowa isolate, solubilized glycoprotein from three *C. hominis* isolates was **screened** for reactivity using Western immunoblots. One of ten P23 MAbs and three of 21

GP900 MAbs were not reactive with any of the three *C. hominis* isolates. The non-reactive P23 MAb binds to a peptide epitope, while the non-reactive GP900 MAbs bind to either carbohydrate/**carbohydrate-dependent** or peptide epitopes of *C. parvum*. These results demonstrate phenotypic differences between *C. hominis* and *C. parvum* within two (glyco)proteins that are involved in parasite gliding motility and attachment/invasion.

L17 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 2  
2007494126. PubMed ID: 17630779. Fine epitope mapping of monoclonal antibody 5F1 reveals anticatalytic activity toward the N domain of human angiotensin-converting enzyme. Danilov Sergei M; Watermeyer Jean M; Balyasnikova Irina V; Gordon Kerry; Kugaevskaya Elena V; Elisseeva Yulia E; Albrecht Ronald F 2nd; Sturrock Edward D. (Department of Anesthesiology, University of Illinois at Chicago, Chicago, Illinois 60612, USA.. danilov@uic.edu) . Biochemistry, (2007 Aug 7) Vol. 46, No. 31, pp. 9019-31. Electronic Publication: 2007-07-14. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Angiotensin I-converting enzyme (ACE, peptidyl dipeptidase, EC 3.4.15.2) is a key enzyme in cardiovascular pathophysiology. A wide spectrum of monoclonal antibodies to different epitopes on the N and C domains of human ACE has been used to study different aspects of ACE biology. In this study we characterized the monoclonal antibody (mAb) 5F1, developed against the N domain of human ACE, which recognizes both the catalytically active and the denatured forms of ACE. The epitope for mAb 5F1 was defined using species cross-reactivity, synthetic peptide (PepScan technology) and phage display library screening, Western blotting, site-directed mutagenesis, and protein modeling. The epitope for mAb 5F1 shows no overlap with the epitopes of seven other mAbs to the N domain described previously and is localized on the other side of the N domain globule. The binding of mAb 5F1 to ACE is **carbohydrate-dependent** and increased significantly as a result of altered glycosylation after treatment with alpha-glucosidase-1 inhibitor, N-butylideneoxynirimycin (NB-DNJ), or neuraminidase. Out of 17 species tested, mAb 5F1 showed strict primate ACE specificity. In addition, mAb 5F1 recognized human ACE in Western blots and on paraffin-embedded sections. The sequential part of the epitope for mAb 5F1 is created by the N-terminal part of the N domain, between residues 1 and 141. A conformational region of the epitope was also identified, including the residues around the glycan attached to Asn117, which explains the sensitivity to changes in glycosylation state, and another stretch localized around the motif 454TPPSRYN460. Site-directed mutagenesis and inhibition assays revealed that mAb 5F1 inhibits ACE activity at high concentrations due to binding of residues on both sides of the active site cleft, thus supporting a hinge-bending mechanism for substrate binding of ACE.

L17 ANSWER 3 OF 5 MEDLINE on STN DUPLICATE 3  
2003351603. PubMed ID: 12695908. Immunodiagnostically applicable monoclonal antibodies to the circulating anodic antigen of *Schistosoma mansoni* bind to small, defined oligosaccharide epitopes. Vermeer H J; van Dam G J; Halkes K M; Kamerling J P; Vliegenthart J F G; Hokke C H; Deelder A M. (Department of Parasitology, Center of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, The Netherlands. ) Parasitology research, (2003 Jul) Vol. 90, No. 4, pp. 330-6. Electronic Publication: 2003-04-15. Journal code: 8703571. ISSN: 0932-0113. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Gut-associated glycoproteins constitute a major group of the circulating excretory antigens produced by human *Schistosoma* species. The O-glycans of the relatively abundant circulating anodic antigen (CAA) from *S. mansoni* carry long stretches of unique -->6(GlcA beta 1-->3)GalNAc beta

1--> repeats. Specific anti-carbohydrate monoclonal **antibodies** (mAbs) are essential tools for the immunodiagnostic detection of CAA in the serum or urine of Schistosoma-infected subjects. In order to define the epitopes recognised by these anti-CAA mAbs, we **screened** a series of protein-coupled synthetic di- to pentasaccharide building blocks of the CAA polysaccharide for immunoreactivity, using ELISA and surface plasmon resonance spectroscopy. It was shown that anti-CAA IgM mAbs preferentially recognise -->6(GlcA beta 1-->3)GalNAc beta 1--> disaccharide units. Interestingly, no mouse anti-CAA mAbs of the IgG class were found that bind to the synthetic epitopes, although many of the IgG mAbs tested do recognise native CAA in a **carbohydrate-dependent** manner. In addition, both IgM and IgG class **antibodies** could be detected in human infection sera using the synthetic CAA fragments. These synthetic schistosome glycan epitopes and their matching set of specific mAbs are useful tools that further the development of diagnostic methods and are helpful in defining the immunological responses of the mammalian hosts to schistosome glycoconjugates.

L17 ANSWER 4 OF 5 MEDLINE on STN DUPLICATE 4  
2000129062. PubMed ID: 10667600. A peptide mimic of E-selectin ligand inhibits sialyl Lewis X-dependent lung colonization of tumor cells. Fukuda M N; Ohyama C; Lowitz K; Matsuo O; Pasqualini R; Ruoslahti E; Fukuda M. (The Burnham Institute, Cancer Research Center, La Jolla, California 92037, USA.. michiko@burnham-inst.org) . Cancer research, (2000 Jan 15) Vol. 60, No. 2, pp. 450-6. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Selectins bind to carbohydrate ligands in a calcium-dependent manner and play critical roles in host defense and possibly in tumor metastasis. To isolate peptides that mimic E-selectin ligands, we **screened** a phage peptide library using E-selectin as a target molecule. This attempt unexpectedly failed, probably because the binding affinity of E-selectin to its ligand is low. We then took an approach that is analogous to the isolation of anti-idiotype **antibodies** and were able to isolate peptides that bound to anticalcbohydrate **antibodies** recognizing E-selectin ligands. These peptides, enriched for their binding to anti-Lewis A **antibody**, were found to bind to E-, P- and L-selectins in a calcium-dependent manner. Phage harboring the identified peptide IELLQAR and synthetic peptides having the same sequence inhibited the binding of sialyl Lewis X or sialyl Lewis A oligosaccharides to E-selectin. The adhesion of HL-60 and B16 melanoma cells expressing sialyl Lewis X to E-selectin was also inhibited by the phage-displaying IELLQAR peptide. Moreover, i.v. injected IELLQAR peptide inhibited the lung colonization of mouse B16 melanoma and human lung tumor cells expressing sialyl Lewis X. These results demonstrate that it is possible to isolate peptides mimicking carbohydrate ligands by **screening** the peptides for binding to anticalcbohydrate **antibodies** and then using them to inhibit **carbohydrate-dependent** experimental tumor metastasis.

L17 ANSWER 5 OF 5 MEDLINE on STN DUPLICATE 5  
1991153563. PubMed ID: 1705525. **Carbohydrate-dependent** epitope mapping of human thyrotropin. Papandreou M J; Sergi I; Benkirane M; Ronin C. (Laboratoire de Biochimie, URA 1179 CNRS, Faculte de Medecine-Secteur Nord, Marseilles, France. ) Molecular and cellular endocrinology, (1990 Oct 1) Vol. 73, No. 1, pp. 15-26. Journal code: 7500844. ISSN: 0303-7207. Pub. country: Netherlands. Language: English.

AB To probe possible effects of carbohydrate chains in the conformation of pituitary glycoprotein hormones, two radiolabeled derivatives of human thyroid-stimulating hormone (hTSH), either partially deglycosylated in the beta-subunit or fully deglycosylated in both the alpha- and beta-subunits, were compared to the native hormone for binding to monoclonal as well as

**polyclonal antibodies.** Monoclonal **antibodies** were **screened** for their ability to bind the intact hormone (anti-hTSH), hTSH and its free alpha-subunit (anti-alpha) or its free beta-subunit (anti-beta). A panel of 14 monoclonal **antibodies** directed against at least eight out of the 12 epitopes known to be present in the hormone was tested in solid-phase assays for their capacity to bind intact and deglycosylated forms of hTSH. All of them displayed identical recognition of native and partially deglycosylated  $^{125}\text{I}$ -hTSH. In contrast, binding of fully deglycosylated  $^{125}\text{I}$ -hTSH to anti-hTSH and anti-beta **antibodies** was dramatically lost while that of anti-alpha was preserved. This clearly indicates that most of the epitopes specific for subunit association as well as those present on the beta-subunit are glycosylation dependent. No alteration was found in **antibody** recognition following deglycosylation of free individual subunits, indicating that the carbohydrate effect can only occur in the combined dimer. Using polyclonal antisera raised against the International Reference Preparations, we found that the deglycosylated hormone could be bound by the anti-beta antiserum although at a much lower dilution than the native antigen, suggesting the presence of at least one glycosylation-independent epitope in the beta-subunit. Competitive binding assays revealed that deglycosylated hTSH is 5 times less immunoreactive toward the anti-beta compared to the anti-alpha antiserum. The current data thus demonstrate the presence of the glycosylation-independent epitopes in the alpha-subunit of hTSH and the localization of most of the glycosylation-dependent domains in the beta-subunit.

```
=> s recombinant human TSH
L18      1145 RECOMBINANT HUMAN TSH

=> s l18 and oversialylated
L19      0 L18 AND OVERSIALYLATED

=> s l18 and asialo recTSH
L20      0 L18 AND ASIALO RECTSH

=> s l18 and sialylation
L21      0 L18 AND SIALYTATION

=> s recombinant human thyrotropin
L22      800 RECOMBINANT HUMAN THYROTROPIN

=> s l22 and oversialylated
L23      0 L22 AND OVERSIALYLATED

=> s l22 and sialylation
L24      20 L22 AND SIALYLATION

=> s l24 and branched
L25      0 L24 AND BRANCHED

=> s l24 and lectin chromatography
L26      0 L24 AND LECTIN CHROMATOGRAPHY

=> s l24 and fucosylation
L27      4 L24 AND FUCOSYLATION

=> dup remove 127
PROCESSING COMPLETED FOR L27
L28      2 DUP REMOVE L27 (2 DUPLICATES REMOVED)
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=> d 128 1-2 cbib abs

L28 ANSWER 1 OF 2 MEDLINE on STN  
2006028258. PubMed ID: 16372382. Characterization of N-glycans of recombinant human thyrotropin using mass spectrometry. Morelle Willy; Donadio Sandrine; Ronin Catherine; Michalski Jean-Claude. (Unite Mixte de Recherche CNRS/USTL 8576, Glycobiologie Structurale et Fonctionnelle, IFR 118, Universite des Sciences et Technologies de Lille 1, 59655 Villeneuve d'Ascq Cedex, France. willy.morelle@univ-lille1.fr) . Rapid communications in mass spectrometry : RCM, (2006) Vol. 20, No. 3, pp. 331-45. Journal code: 8802365. ISSN: 0951-4198. Pub. country: England: United Kingdom. Language: English.

AB Thyroid-stimulating hormone is a vital component of the regulatory mechanism that maintains the structure and function of the thyroid gland and governs thyroid hormone release. In this paper we report the first detailed structural characterization of the N-linked oligosaccharides of recombinant human thyroid-stimulating hormone (rhTSH). Using a strategy combining mass spectrometric analysis and sequential exoglycosidase digestion, we have defined the structures of the N-glycans released from recombinant human thyrotropin by peptide N-glycosidase F. All glycans are complex-type glycans and are mainly of the bi- and triantennary type with variable degrees of fucosylation and sialylation. The major non-reducing epitope in the complex-type glycans is: NeuAcalpha2-3Galbeta1-4GlcNAc (sialylated LacNAc). The carbohydrate microheterogeneity at the three glycosylation sites was studied using reversed-phase high-performance liquid chromatography (RP-HPLC), concanavalin A affinity chromatography and mass spectrometric techniques, including both matrix-assisted laser desorption/ionization (MALDI) and electrospray. rhTSH was reduced, carboxymethylated and then digested with trypsin. The mixture of peptides and glycopeptides was subjected to RP-HPLC and the structures of the glycopeptides were determined by MALDI in conjunction with on-target exoglycosidase digestions. After PNGase F digestion, the peptide moiety of the glycopeptide was determined by the presence of the b- and y-series ions derived from its amino acid sequence in the quadrupole time-of-flight tandem mass (QTOF-MS/MS) spectrum. Glycosylation sites Asn-alpha52 and Asn-alpha78 contain mainly bi- and triantennary complex-type glycans. Only glycosylation site Asn-alpha52 bears fucosylated N-glycans. Minor tetraantennary complex structures were also observed on both glycosylation sites. Profiling of the carbohydrate moieties of Asn-beta23 indicates a large heterogeneity. Bi-, tri-, and tetraantennary N-glycans were present at this site. These data demonstrate site-specificity of glycosylation in the alpha subunit but not in the beta subunit of rhTSH with Asn-alpha52 bearing essentially di- and triantennary glycans with or without core fucosylation and bi- and triantennary glycans with no core fucosylation being attached to Asn-alpha78.

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L28 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2009 ACS on STN  
2006:153674 Document No. 144:382215 Characterization of N-glycans of recombinant human thyrotropin using mass spectrometry. Morelle, Willy; Donadio, Sandrine; Ronin, Catherine; Michalski, Jean-Claude (Unite Mixte de Recherche CNRS/USTL 8576, "Glycobiologie Structurale et Fonctionnelle", Universite des Sciences et Technologies de Lille 1, Villeneuve d'Ascq, 59655, Fr.). Rapid Communications in Mass Spectrometry, Volume Date 2006, 20(3), 331-345 (English) 2005. CODEN: RCMSEF. ISSN: 0951-4198. Publisher: John Wiley & Sons Ltd..

AB TSH is a vital component of the regulatory mechanism that maintains the structure and function of the thyroid gland and governs thyroid hormone release. In this paper the authors report the first detailed structural characterization of the N-linked oligosaccharides of recombinant human TSH

(rhTSH). Using a strategy combining mass spectrometric anal. and sequential exoglycosidase digestion, the authors have defined the structures of the N-glycans released from recombinant human TSH by peptide N-glycosidase F. All glycans are complex-type glycans and are mainly of the bi- and triantennary type with variable degrees of **fucosylation** and **sialylation**. The major non-reducing epitope in the complex-type glycans is: NeuAc $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc (sialylated LacNAc). The carbohydrate microheterogeneity at the three glycosylation sites was studied using reversed-phase HPLC (RP-HPLC), Con A affinity chromatog. and mass spectrometric techniques, including both matrix-assisted laser desorption/ionization (MALDI) and electrospray. rhTSH was reduced, carboxymethylated and then digested with trypsin. The mixture of peptides and glycopeptides was subjected to RP-HPLC and the structures of the glycopeptides were determined by MALDI in conjunction with on-target exoglycosidase digestions. After PNGase F digestion, the peptide moiety of the glycopeptide was determined by the presence of the b- and y-series ions derived from its amino acid sequence in the quadrupole time-of-flight tandem mass (QTOF-MS/MS) spectrum. Glycosylation sites Asn- $\alpha$ 52 and Asn- $\alpha$ 78 contain mainly bi- and triantennary complex-type glycans. Only glycosylation site Asn- $\alpha$ 52 bears fucosylated N-glycans. Minor tetraantennary complex structures were also observed on both glycosylation sites. Profiling of the carbohydrate moieties of Asn- $\beta$ 23 indicates a large heterogeneity. Bi-, tri-, and tetraantennary N-glycans were present at this site. These data demonstrate site-specificity of glycosylation in the  $\alpha$  subunit but not in the  $\beta$  subunit of rhTSH with Asn- $\alpha$ 52 bearing essentially di- and triantennary glycans with or without core **fucosylation** and bi- and triantennary glycans with no core **fucosylation** being attached to Asn- $\alpha$ 78.

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=> s 122 and antibod?
L29      152 L22 AND ANTIBOD?

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L30      6 L29 AND SCREEN?

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PROCESSING COMPLETED FOR L30
L31      4 DUP REMOVE L30 (2 DUPLICATES REMOVED)
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=> d 131 1-4 cbib abs
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L31 ANSWER 1 OF 4      MEDLINE on STN
2006218519. PubMed ID: 16624113. The relationship between serum thyroid autoantibodies, iodine intake, development and prognosis of Graves' disease. Chen Wei; Man Na; Li Yu-shu; Shan Zhong-yan; Teng Wei-ping. (Endocrinology Department, Endocrinology Institute, The First Affiliated Hospital of China Medical University, Shenyang 110001, China. ) Zhonghua nei ke za zhi [Chinese journal of internal medicine], (2006 Feb) Vol. 45, No. 2, pp. 95-9. Journal code: 16210490R. ISSN: 0578-1426. Pub. country: China. Language: Chinese.
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AB OBJECTIVE: To investigate the relationship of thyroid autoantibodies including serum thyroid stimulating **antibody** (TSAb), thyroid stimulation blocking **antibody** (TSBAb) and iodine intake with the development and prognosis of Graves' hyperthyroidism. METHODS: A total of 63 subjects with overt hyperthyroidism were **screened** out from 3 Chinese rural communities with different iodine intakes at first survey. Serum TSAb, TSBAb, thyrotropin binding inhibitory immunoglobulin (TBII), thyroid peroxidase **antibody** (TPOAb) and thyroglobulin **antibody** (TGAb) were detected. The patients were followed up 2 years later. TSAb and TSBAb were measured with **recombinant**

human thyrotropin receptor (rhTSHR)-Chinese hamster ovary cell (rhTSHR-CHO cell) bioassay. RESULTS: At the first survey, the prevalences of positive TSAb, TBII and TSBAb were found in 80.9%, 61.7% and 6.4% of the patients with Graves' disease respectively. TSAb and/or TBII were positive in 91.5% of the patients. The consistent rate of TSAb and TBII was 59.6% in the cases. All indexes mentioned above were higher in the patients than in healthy controls. Positive correlations were found between TSAb and TBII ( $r = 0.407$ ), TSAb and thyroglobulin ( $r = 0.301$ ), TSAb and thyroid volume ( $r = 0.317$ ) respectively. The prevalence of positive TSAb (91.7%) in Graves' patients in iodine excessive area are significantly higher than those in iodine mildly deficient area (66.7%). The positive rates and the titers of TBII, TPOAb and TGAb were not different statistically among the patients in the three communities. At follow-up, the patients with Graves' hyperthyroidism were classified into euthyroid group (G1) and hyperthyroid group (G2) according to their outcomes of the disease. The TSAb titers and the thyroid volume in the cases of G1 decreased significantly, whereas the patients with highly positive TPOAb titers in the first survey and the follow-up were hard to become euthyroid and TSAb may be the secondary factor influencing the thyroid as compared with TPOAb. CONCLUSION: TSAb is more significant than TBII in diagnosing and predicting the outcomes of Graves' hyperthyroidism. The application of both TSAb and TBII could raise the positive rates of thyrotrophin receptor **antibody** tests. TSAb, TPOAb titers and thyroid volume were factors influencing the prognosis of Graves' hyperthyroidism.

L31 ANSWER 2 OF 4 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

2004:38440 The Genuine Article (R) Number: 756YR. Three-week thyroxine withdrawal thyroglobulin stimulation **screening** test to detect low-risk residual/recurrent well-differentiated thyroid carcinoma. Walish P G (Reprint). Mt Sinai Hosp, Endocrine Unit, Rm 782, 600 Univ Ave, Toronto, ON M5G 1X5, Canada (Reprint). Golger A; Fridman T R; Eski S; Witterick I J; Freeman J L. Mt Sinai Hosp, Dept Otolaryngol, Toronto, ON, Canada; Mt Sinai Hosp, Dept Med, Toronto, ON, Canada; Univ Toronto, Med Sch, Toronto, ON, Canada.

JOURNAL OF ENDOCRINOLICAL INVESTIGATION (OCT 2003) Vol. 26, No. 10, pp. 1023-1031. ISSN: 0391-4097. Publisher: EDITRICE KURTIS S R L, VIA LUIGI ZOJA 30, 20153 MILAN, ITALY. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Measurement of serum TSH-stimulated thyroglobulin (Tg) is recognized as a sensitive method for detecting residual/recurrent well-differentiated thyroid carcinoma (WDTC) in patients previously treated by surgery and radioactive iodine (RAI) ablation therapy. WDTC patients who have an undetectable serum Tg on thyroid hormone therapy (THT) in the absence of Tg-**antibody** interference are considered to be at low risk for residual/recurrent disease. Traditional management has been to withdraw T-4 for 4-6 weeks or T-3 for 2 weeks to stimulate endogenous TSH. However, this prolonged THT withdrawal induces hypothyroidism and its concomitant morbidity. In the present study, we assess the efficacy of shortening the time of T-4 withdrawal to only 3 weeks for detecting residual/recurrent WDTC as a sufficient serum TSH stimulus for obtaining a positive serum Tg result without a routine diagnostic whole body scan (WBS). Additionally, we have evaluated the impact of such a T-4 withdrawal interval on quality of life and loss of employment time. A total of 181 patients with WDTC selected for study had previously been treated with a bilateral surgical thyroidectomy followed by RAI ablation therapy (average post-surgery to follow-up interval of 10.8 yr). All of the cohort had an undetectable ( $<1 \text{ mug/l}$ ) serum Tg on THT without Tg-**antibody** interference. Serum TSH and Tg were measured before and after cessation of T-4 therapy for 3 weeks. A serum Tg greater than or equal to  $2 \text{ mug/l}$  was considered positive for residual/recurrent disease. A

quality of life questionnaire [Short-Form 36 (SF-36)] was administered before withdrawal, at peak TSH and after resumption of therapy. From the completed SF-36 questionnaires, the overall degree of functional impairment was not severe and did not result in loss of employment time. Moreover, this protocol identified three possible responses to the 3-week T-4 withdrawal interval as follows: a) serum Tg undetectable with TSH 25 mIU/l (similar to 75% of total cohort); b) serum Tg greater than or equal to 2 mug/l (similar to 10% of total cohort) which will require further investigation and treatment for residual/recurrent disease; c) undetectable serum Tg with inadequate TSH rise (similar to 15% of total cohort), which will require TSH stimulation by either longer T-4 withdrawal or recombinant human TSH to exclude residual disease. We conclude that a stimulated serum Tg test performed 3 weeks after T-4 withdrawal is a simple and cost-effective first-line screening test with minimal morbidity which is sufficient to evaluate low-risk WDTC patients for recurrent/residual carcinoma. (C) 2003, Editrice Kurtis.

L31 ANSWER 3 OF 4 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN  
2002270409 EMBASE Management of low-risk well-differentiated thyroid cancer based only on thyroglobulin measurement after **recombinant human thyrotropin**. Wartofsky, Leonard, Dr. (correspondence). Department of Medicine, Washington Hospital Center, 110 Irving Street, N.W., Washington, DC 20010-2975, United States. Leonard.wartofsky@medstar.net. Thyroid Vol. 12, No. 7, pp. 583-590 2002. Refs: 20. ISSN: 1050-7256. CODEN: THYRER. Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 20020815. Last Updated on STN: 20020815

AB A multicenter study was undertaken to ascertain prevalence and significance of **recombinant human thyrotropin** (rhTSH)-stimulated increases in thyroglobulin (Tg) levels in thyroid cancer patients classified to be at low risk for recurrence. Patients were eligible for enrollment if they had undergone near-total or total thyroidectomy and remnant ablation between 1-10 years prior to enrollment and had received thyroxine suppression therapy (THST) with a TSH level of < 0.5 mU/L and Tg level less than or equal to 5 ng/mL within the prior year. Patients with anti-Tg **antibodies**, distant metastases, or other evidence of residual disease were excluded. Four hundred eighty-six patients were entered into the study, and 300 were considered eligible and comprise the study population. TSH, Tg, and anti-Tg **antibody** levels were obtained at baseline, followed by intramuscular injection of 0.9 mg of rhTSH on days 1 and 2 and measurement of Tg on day 5. After rhTSH, 53 patients (18%) had elevations in Tg of at least 2 ng/mL, including 33 patients (11%) with increases from baseline of equal to or greater than 5 ng/mL. Patients with an initial advanced stage of disease were more likely to display elevations in Tg after rhTSH. One third of those with stage III disease displayed elevations in Tg of 2 ng/mL or more. Patients within 5 years of thyroidectomy were as likely to display elevations in rhTSH-stimulated Tg as those 5-10 years from surgery. In conclusion, these data suggest rhTSH-stimulated Tg testing without scan may be a useful tool in the follow-up of patients with low-risk thyroid cancer, and may serve to identify patients previously thought free of disease on the basis of undetectable Tg levels while undergoing THST. A strategy is presented for incorporation of this approach into the management of patients with low-risk well-differentiated thyroid cancer.

L31 ANSWER 4 OF 4 MEDLINE on STN  
1998241859. PubMed ID: 9580758. Bioassay of thyrotropin receptor **antibodies** with Chinese hamster ovary cells transfected with **recombinant human thyrotropin** receptor:

DUPLICATE 1

clinical utility in children and adolescents with Graves disease. Botero D; Brown R S. (Department of Pediatrics, University of Massachusetts Medical School, Worcester, USA.) The Journal of pediatrics, (1998 Apr) Vol. 132, No. 4, pp. 612-8. Journal code: 0375410. ISSN: 0022-3476. Pub. country: United States. Language: English.

AB OBJECTIVE: The objective of this study was to compare the clinical utility of a new bioassay for thyrotropin (TSH) receptor **antibodies** (Abs) with the conventional radioreceptor assay and with measurement of thyroid peroxidase Abs in the diagnosis of Graves disease in childhood. STUDY DESIGN: Serum samples obtained from 22 children and adolescents with Graves disease (19 hyperthyroid, 3 in remission), 13 children and adolescents with chronic lymphocytic thyroiditis, and 17 normal children in a control group were evaluated. RESULTS: TSH receptor Abs were detected by bioassay in 10 (91%) of 11 patients with active Graves disease but in 0 of 2 patients in remission, 0 of 13 normal members of the control group, and 0 of 11 patients with chronic lymphocytic thyroiditis including 1 with thyrotoxicosis. The sensitivity and specificity of TSH receptor Abs detected by radioreceptor assay studied in the same 11 patients and in an additional 11 patients was similar to bioassay. In contrast, thyroid peroxidase Abs were detected in only 12 (71%) of 17 patients with Graves disease but in 11 of 11 patients with chronic lymphocytic thyroiditis and in 0 of 17 members of the control group. CONCLUSION: Bioassay of TSH receptor Abs is both sensitive and specific for the diagnosis of active Graves disease in the young. When cost and simplicity are considered, however, bioassay offers no advantage over radioreceptor assay for initial diagnostic **screening**. Rather, bioassay for TSH receptor Abs may be useful in thyrotoxic patients who are negative initially in the radioreceptor assay or in treated patients whose clinical picture is discordant with results in the radioreceptor assay.

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L33          24 L22 AND CHROMATOGRAPHY

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L35          0 L33 AND LENTIL

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L39          5 DUP REMOVE L38 (0 DUPLICATES REMOVED)

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L39  ANSWER 1 OF 5      MEDLINE on STN
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1999299935. PubMed ID: 10372720. Analysis of carbohydrate residues on recombinant human thyrotropin receptor. Oda Y; Sanders J; Roberts S; Maruyama M; Kiddie A; Furmaniak J; Smith B R. (FIRS Laboratories, RSR Ltd., Llanishen, Cardiff, United Kingdom. ) The Journal of clinical endocrinology and metabolism, (1999 Jun) Vol. 84, No. 6, pp. 2119-25. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB An investigation of the sugar groups on recombinant human TSH receptors (TSHR) expressed in CHO-K1 cells and solubilized with detergents is described. Western blotting studies with TSHR monoclonal antibodies showed that the receptor was present principally as two bands with approximate molecular masses of 120 and 100 kDa. Further blotting studies using lectins and/or involving treatment with different glycosidases indicated that the 100-kDa band contained about 16 kDa of high mannose-type sugars, and the 120-kDa band contained about 33 kDa of complex-type sugars. It was possible to separate the 120- and 100-kDa components of the TSHRs by lectin affinity chromatography. In particular, *Galanthus nivalis* lectin, which binds high mannose-type sugars, bound the 100-kDa band, but not the 120-kDa band, whereas *Datura stramonium* lectin, which binds complex-type sugars, bound the 120-kDa band, but not the 100-kDa band. <sup>125</sup>I-Labeled TSH binding studies with the various lectin column fractions showed that TSH-binding activity was principally associated with the complex-type sugar containing the 120-kDa form of the receptor rather than the high mannose-containing 100-kDa form. During peptide chain glycosylation, high mannose-type sugar residues are attached first and then modified by the formation of complex type structures to form the mature glycoprotein. Our data suggest that in the case of the TSH receptor, this type of posttranslational processing has an important role in forming the TSH-binding site.

L39 ANSWER 2 OF 5 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

1998:689498 The Genuine Article (R) Number: 117GC. Modulation of human thyrotropin oligosaccharide structures - enhanced proportion of sialylated and terminally galactosylated serum thyrotropin isoforms in subclinical and overt primary hypothyroidism.

Schaaf L (Reprint). Max Planck Inst Psychiat, Kraepelinstr 10, D-80804 Munchen, Germany (Reprint). Trojan J; Theodoropoulou M; Usadel K H; Stalla G K. Max Planck Inst Psychiat Endokrinol & Klin Chem, D-80804 Munchen, Germany; Univ Frankfurt Klinikum, Zentrum Inneren Med, D-60590 Frankfurt, Germany.

JOURNAL OF ENDOCRINOLOGY (SEP 1998) Vol. 158, No. 3, pp. 359-365. ISSN: 0022-0795. Publisher: SOC ENDOCRINOLOGY, 17/18 THE COURTYARD, WOODLANDS, BRADLEY STOKE, BRISTOL BS32 4NQ, ENGLAND. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Enhanced sialylation of thyrotropin (TSH) prolongs its metabolic clearance rate and thus increases the hormone's *in vivo* bioactivity. This has been shown for hypothyroid rats and for recombinant human TSH, but there are few data on the sialylation of human serum TSH. The aim of this work was to further study sialylated human serum TSH, its precursors bearing terminal galactose residues, and the role of pharmacological doses of thyrotropin-releasing hormone (TRH) on their secretion under different degrees of primary hypothyroidism.

We analyzed serum TSH in patients with subclinical (n=9) and overt primary hypothyroidism (n=13) compared with euthyroid individuals (n=12) and human standard pituitary TSH (IRP 80/558). Blood was drawn before and 30 min after intravenous administration of 200 mU g TRH, and TSH was purified by immunoaffinity concentration. The content of sialylated (sialo-) TSH and isoforms bearing terminal galactose (Gal-TSH, asialo-Gal-TSH) was measured by *Ricinus communis* (RCA 120) affinity chromatography in combination with enzymatic cleavage of sialic

acid residues. TSH immunoreactivity was measured by an automated second generation TSH immunoassay.

Pituitary TSH contained  $16.5 \pm 0.8\%$  Gal-TSH. In euthyroid individuals the proportion of Gal-TSH was  $14.6 \pm 9\%$ , whereas TSH in patients with subclinical and overt primary hypothyroidism contained  $23.9 \pm 3.5\%$  ( $P < 0.05$  vs euthyroid individuals) and  $21.1 \pm 1.7\%$  Gal-TSH respectively. The mean ratio of asialo-Gal TSH was  $23.8 \pm 0.6\%$  for pituitary TSH,  $35.7 \pm 4.2\%$  in euthyroid individuals,  $48.0 \pm 3.3\%$  in patients with subclinical, and  $61.5 \pm 3.8\%$  ( $P < 0.001$  vs euthyroid individuals) in patients with overt primary hypothyroidism. For pituitary TSH the calculated proportion of sialo-TSH was  $65 \pm 0.2\%$ , for euthyroid individuals  $20.3 \pm 2.8\%$ , for patients with subclinical hypothyroidism  $24.1 \pm 3.0\%$ , and for patients with overt primary hypothyroidism  $40.7 \pm 3.0\%$  ( $P < 0.001$  vs euthyroid individuals). The proportions of Gal-TSH, asialo-Gal-TSH, and sialo-TSH did not differ significantly before and after TRH administration in the individuals studied.

Our data show that patients with subclinical and overt primary hypothyroidism have a markedly increased proportion of serum TSH isoforms bearing terminal galactose and sialic acid residues, which may represent a mechanism for the further stimulation of thyroid function. Pharmacological doses of TRH cause an increased quantity of TSH to be released, but do not significantly alter the proportion of sialylated or terminally galactosylated TSH isoforms.

L39 ANSWER 3 OF 5 MEDLINE on STN  
1996368590. PubMed ID: 8772598. Large scale synthesis of recombinant human thyrotropin using methotrexate amplification: chromatographic, immunological, and biological characterization. Hussain A; Zimmerman C A; Boose J A; Froehlich J; Richardson A; Horowitz R S; Collins M T; Lash R W. (Department of Medicine, University of Maryland School of Medicine, Baltimore, USA.) The Journal of clinical endocrinology and metabolism, (1996 Mar) Vol. 81, No. 3, pp. 1184-8. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB Studies of human TSH (hTSH) structure and function have been limited by difficulties in producing large quantities of recombinant hormone. We describe a system for the stable expression of high levels of recombinant human TSH (rec hTSH) using a mutant form of dihydrofolate reductase (dhfr) as an amplifiable dominant selectable marker. A vector expressing both the hTSH alpha-subunit and the mutant dhfr was cotransfected with a hTSH beta-subunit expression vector into dhfr-deficient cells. Amplification of the transfected sequences by methotrexate selection, followed by cell culture in a hollow fiber perfusion system, yielded rec hTSH production as high as 100,000 microU/ mL. Immunoradiometric assays using five different antibodies revealed no differences in the immunological activities of rec hTSH and pituitary hTSH. Biacactivity was measured in a novel TSH bioassay coupling the generation of cAMP by a transfected hTSH receptor to the cAMP-dependent regulation of a luciferase reporter gene. The ED50 for bovine TSH in this bioassay was 1.4 ng/mL ( $3.5 \times 10^{-11}$  mol/L). The ratio of the ED50 values for rec hTSH and pituitary hTSH was 1.0:1.1 ( $P = NS$ ), indicating that the two TSHs were of equivalent potency. In conclusion, we have developed techniques for the high level production of rec hTSH that is immunologically and biologically equivalent to pituitary hTSH. The ability to produce large quantities of rec hTSH using standard laboratory techniques should facilitate future studies, such as the development of clinically useful TSH analogs.

L39 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN  
1996:683559 The Genuine Article (R) Number: VH267. Glycosylation is the structural basis for changes in polymorphism and immunoreactivity of pituitary glycoprotein hormones.

Zerfaoui M (Reprint); Ronin C. UPR 9024 CNRS, GLM, F-13402 MARSEILLE 20, FRANCE.

EUROPEAN JOURNAL OF CLINICAL CHEMISTRY AND CLINICAL BIOCHEMISTRY (SEP 1996) Vol. 34, No. 9, pp. 749-753. ISSN: 0939-4974. Publisher: WALTER DE GRUYTER & CO, GENTHINER STRASSE 13, D-10785 BERLIN, GERMANY. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Glycoprotein hormones have long been known to display extensive polymorphism and changes in bioactivity according to the endocrine status of the patient. Structural analysis has shown that pituitary gonadotropins (lutropin and follitropin) and thyrotropin are synthesized and secreted as a panel of isoforms which differ in glycosylation, bioactivity and circulatory half-life. Ultrasensitive immunoassays could reveal that glycosylation of plasma hormones is structurally different from the pituitary stock so that the ratio of circulating glycoforms may vary according to the physiopathology of the pituitary axis. However, contradictory results between immunoassays have been often reported, suggesting that some plasma forms can escape recognition by monoclonal antibodies which have been raised to the pituitary or urinary antigen. When hormone levels do not correlate with clinical features, one can also suspect that inactive or hyperactive forms are being measured. At the molecular level, very limited information has been gained toward the expression of hormone epitopes as a function of carbohydrate structure. To address this issue, we have compared the recognition of pituitary and recombinant human thyrotropin by various polyclonal and monoclonal antibodies before and after neuraminidase treatment. Both, pituitary and recombinant thyrotropin bound to anti-alpha and anti-beta antibodies, demonstrating thereby that recombinant thyrotropin can be used to calibrate immunoassays. While removal of sialic acid did not alter the recognition of the recombinant hormone in various immunoassays, this treatment specifically abolished the binding of pituitary thyrotropin to anti-beta monoclonal antibodies. These findings show that immunoreactivity of circulating hormone glycoforms, which are often more sialylated than their pituitary counterparts, may very well account for variation depending on the antibodies used in the immunoassays.

L39 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2009 ACS on STN

1996:48889 Document No. 124:76675 Original Reference No. 124:14060h,14061a

The use of recombinant human thyrotropin produced by Chinese Hamster Ovary cells for the preparation of immunoassay reagents. Ribela, Maria Teresa C. P.; Bianco, Antonio C.; Bartolini, Paolo (Department of Application of Nuclear Techniques in Biological Sciences, National Nuclear Energy Commission, Sao Paulo, Brazil). Journal of Clinical Endocrinology and Metabolism, 81(1), 249-56 (English) 1996. CODEN: JCCEMZ. ISSN: 0021-972X. Publisher: Endocrine Society.

AB Recombinant human TSH (rec-hTSH; Thyrogen, lot M-17073) obtained from transformed Chinese hamster ovary cells was tested for both radioiodination and preparation of a secondary standard used in RIA and immunoradiometric assay (IRMA) for routine clin. investigation. Results were compared to those obtained with high quality pituitary TSH (pit-hTSH; Dr. P. Torjesen, Oslo, Norway; and NIDDK, Rockville MD), traditionally used in these assays. After extensive characterization and testing, it was found that [<sup>125</sup>I]rec-hTSH matched all binding and chromatog. critical usually obtained with [<sup>125</sup>I]pit-hTSH, including Stokes' radius, labeling, and storage stability, and did not introduce any significant bias when used in the measurement of unknown serum samples. A preparation of rec-hTSH was calibrated against a local secondary standard as well as against two well known international reference preps. (NIDDK hTSH RP-1 and WHO International Reference Preparation 80/558) by IRMA and RIA. In the RIA, NIDDK anti-hTSH-3 polyclonal antibody was used, whereas in the IRMA, two com.

preps. were used: a monoclonal antibody as the capture antibody. In both assays, the recombinant standard preparation yielded good fit displacement curves, showing significant parallelism compared to pit-hTSH and therefore allowing an unbiased measurement of unknown serum samples. The specific activity of the rec-hTSH preparation calibrated against the WHO International Reference Preparation was 7.7 IU/mg protein when measured by IRMA and 71 IU/mg when measured by RIA. In conclusion, these results indicate for the first time that rec-hTSH can fully replace pit-hTSH as both standard and tracer in diagnostic in vitro systems such as RIA and IRMA, suggesting that other recombinant glycosylated hormones might also serve for immunoassay reagent preparation

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FULL ESTIMATED COST	186.51	186.73
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-4.92	-4.92

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